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SCREENING AND COMPARISON OF IN-VITRO ANTI HELMENTHIC ACTIVITYOF INDIVIDUAL CURCUMINOIDS WITH CHLOROFORM EXTRACT

N.Sanjeevaiah*, P.Pranaya, B.Padmaja, G.Sandhyarani
Department of Pharmacology, Vaageswari College of Pharmacy, Near LMD Police Station,
Thimmapur, Karimnagar-Andhra Pradesh-India.
Email: nagurla.sanjeev@gmail.com

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Abstract

The anthelmintic activity of chloroform extracts of rhizomes of curcuma longa Linn. Were evaluated separately on Indian adult earth worm's pheretima posthuma (annelid) for this work the rhizomes were extracted separately with chloroform and distilled water following by percolation method. Various concentrations (10, 50,100,200mg/ml) of each extract were tested for anthelmentic activity which involved the determination of time of paralysis and time of death of the worms. The curcuma longa crude extract showing the presence of curcuminoids(curcumin, Demethoxy curcumin,Bisdemethoxy curcumin) .All extract were able to show anthelmintic activity at all concentrations. The activities are well comparable with the standard drug, Albendazole as positive control. All the chloroform extracts showed better anthelmintic activity comparable with that of the standard drug. 10% Propylene glycol in normal saline was used as negative control, did not showed any anthelmintic activity.

Key words: Anthelmintic activity, Curcuma longa, Chloroform extract, Albendazole.

Introduction

The prevalence of intestinal helmenthiasis is apparently very high, and on a global scale the infections cause severe health problems in man and domestic animals. More than billon people are infected with ASCARIS LUMBRICOIDS, and hundreds of millions are infected with hookworms and trichuris (guyatt and Evans, 1992).these infections cause intestinal disorders, discomfort and loss of productivity through direct or indirect interference with host nutrition and metabolism.

In live stock, gastrointestinal parasitic infections constitute a major obstacle to animal production all over the world, particularly in tropical and sub tropical areas. The infections cause high economic losses in the form of impaired performance and reproduction, and sometimes significant weight losses and mortality rates (Faviyi et al, 1986). A

number of control measures to combat these infections are available, and several classes of modern synthetic anthelmintics have been shown to a very effective when used strategically in the right epidemiological contest. However, increasing problems of development of resistance in helminthes (Geerts and dorny, 1995; Coles, 1997) against anthelemintic have led to the proposal of screening medical plants for their anthelmintic activities. These plants are known to provide a rich source of botanical anthelminitics (Satyavati et al, 1976).

A number of medicinal plants have been used to treat parasitic infections men and animals. Latex collected from young papaya fruits were shown to process anhelmentic activity against *Ascardia galli* infections in chickens and *Helmosomoids polygrus* in mice. The uses of Calotropis procera flowers as anthelmintics properties offer an alternative to manufactured anthelmintics that is both sustainable and environmentally acceptable .Such plant could have a more important role in the future control of helminth infections in the tropics. With this view, the curcumalanga is studied for its anthelmintic property against annelida

Helmentheasis is prevalent globally(1/3 of world's population harbors them)but is more comman in developing countries with poorer personal hygiene .Multiple infections in the same individual are not uncommon .In the human body, the GIT is the abode of many helminthes ,but some also live in tissues, or there larve migrate into tissues. they harm the host by depriving him of his food ,causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins .Helmentheasis is rarely fatal, but is a major cause of ill health.Anthelmentics or drugs that either that kill(vermicide) or expel(vermifuge) infesting helminthes.

In live stock, gastrointestinal parasitic infections constitute obstacle to animal production all over the world, particularly in tropical and subtropical areas. The infections cause high economic losses in the form of impaired performance and reproduction, and sometimes significant weight losses and mortality rates (Fabiyi et al, 1986)

Materials and Methods:

Drugs and Chemicals: Albandazole (Gift sample obtained from Hetero drugs Pvt.Ltd,A.P),Chloroform(Merck Pvt.Mumbai) Earth worms (Pheretima posthuma) of nearly equal size 8cm were collected from herbal garden,Kakatiya University,Warangal,Andhrapradesh.And other chemicals were procured from local suppliers.

2.1 Collection of Plant material: The rhizomes of *Curcuma longa L.* was collected from different regions of Chittoor District, Andhra Pradesh, India. The plant specimen was authenticated by Dr.K. Madhava chetty, Assistant Professor, Department of botany, Sri Venkateshwara University, Tirupati.

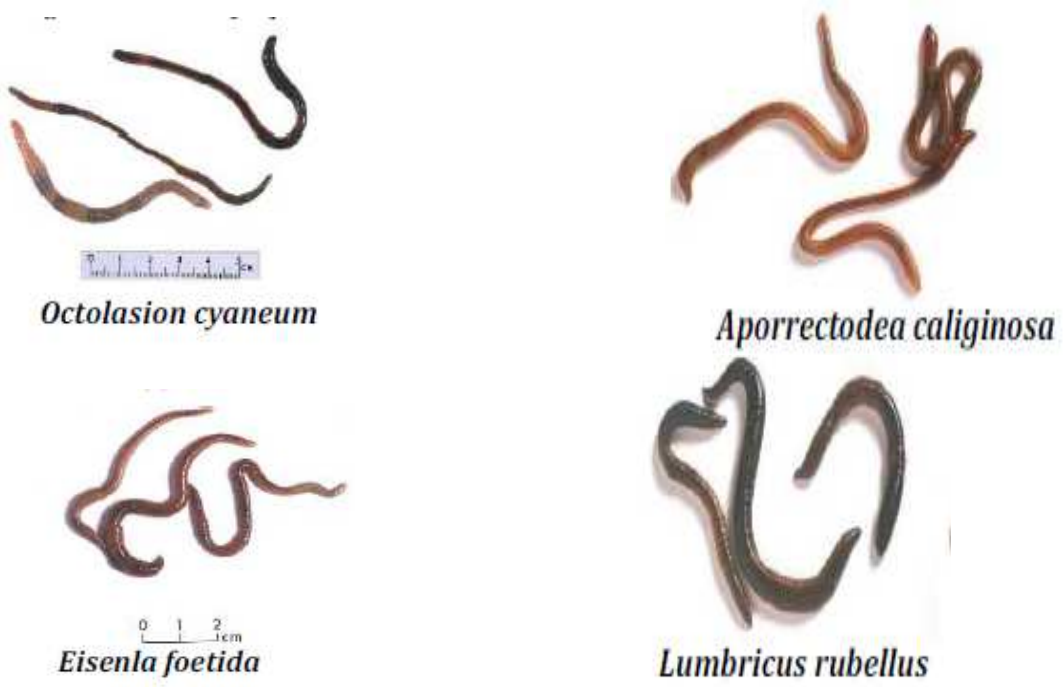
2.3 Preparation of Plant Extract:

The rhizomes were dried in shade and coarsely powdered with a blender.1kg of the powder was subjected to continuous percolation using 5litere Round bottom flask with the solvent chloroform for 24hrs, the solvent was recovered by distillation in rotary vacuum evaporator at 80^0c. 46 gms of chloroform extract was Loaded in column chromatography, eluted with chloroform; methanol (95:5), the residue obtained was characterized by TLC, HPLC, then the above residue was subjected to purification. Eluted with 100%MDC (Methelene dichloride). Three compounds were isolated & characterized by TLC, NMR, &IR the residue was stored in a desicator and used in further studies.

Animals

Healthy adult Indian earthworms *Pheretima posthuma* were used for evaluating the anthelmintic activity due to its anatomical and physiological resemblance with intestinal round worm parasites of human beings.

Figure 1: Different types of worms causing helminthiasis



All healthy earthworms were of approximately 8 cm in size. They were collected from local place, washed and kept in water until they are used for screening of activity.

Anthelmintic activity:

6 worms each i.e.Pheretima posthuma of approximately equal size were taken in petridishes containing 25 ml of each of the Extract solution concentrations, standard drug (Albendazole 10mg/ml) and the control solution. The time

taken by the worms to undergo paralysis i.e. no movement unless shaken vigorously, was noted. Also the time of death was recorded when the worms no longer moved when shaken vigorously or even when dipped in hot water. The time taken for paralysis and death of Pheretima posthuma in the standard solution and the various concentrations of the test extracts are tabulated in Table1 and represented in Figure 1 & 2.

Results and Discussion:

Table1: **Time Taken for Paralysis and Death in Chloroform Extract, Isolated Compounds Treated Groups and Albendazole.**

Group	Solution	Concentration (mg/ml)	Time taken for paralysis(min)	Time taken for death (min)
1	Control solution	-	-	-
2	Albendazole	10	38±0.03	56±0.3
3	Chloroform Extract	10	17.5±0.2	32±0.3
		50	31.3±0.3	26.5±0.1
		100	11.7±0.2	17.3±0.2
		200	9.2±0.2	15.3±0.4
4	Curcumin	10	15.6±0.2	31±0.3
		50	11.7±0.1	24±0.2
		100	8.5±0.2	15±0.4
		200	6.2±0.3	12±0.1
5	Demethoxy curcumin	10	14.7±0.4	30±0.3
		50	10.5±0.1	22±0.1
		100	7.3±0.3	13±0.3
		200	5.1±0.2	10±0.4
6	Bisdemethoxy curcumin	10	12.7±0.1	27±0.2
		50	9.5±0.3	18.1±0.1
		100	6.1±0.4	11.5±0.2
		200	3.2±0.2	7.2±0.4

Figure-2:Time taken for Paralysis in Chloroform Extract, Isolated compounds treated groups and Albendazole.

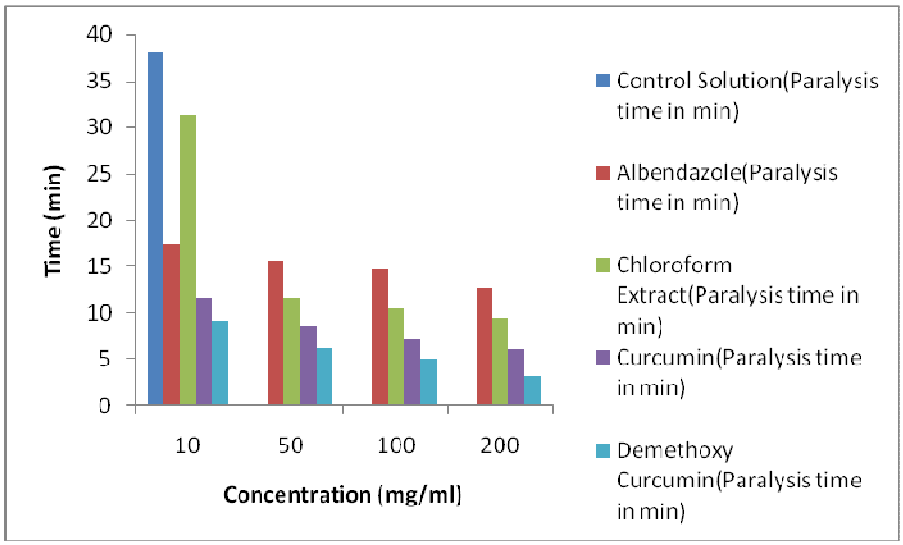


Figure-3: Time taken for death in Chloroform Extract, Isolated compounds treated groups and Albendazole.

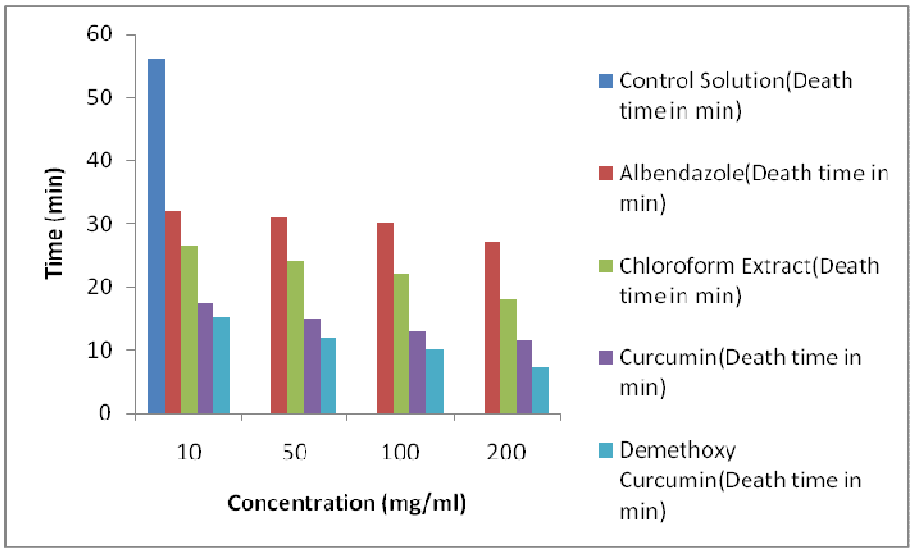


Figure-4: Anthelmintic activity (paralysis) of chloroform extracts of *curcuma longa* L. rhizomes.

10mg/ml 50mg/ml

100mg/ml

200mg/ml

Figure-5: Anthelmintic activity (death) of chloroform extracts of *curcuma longa* L. rhizomes.

10mg/ml

50mg/ml

100mg/ml

200mg/ml

The chloroform extract showed a dose dependent decrease in time taken for paralysis and death of Pheretima posthuma .The maximum dose of 200 mg/ml of chloroform extract, isolated compounds showed the least time taken for paralysis and death of the earth worms. The effect of extract & isolated compounds on the paralysis or helmenthiasis of the worm, according to the result may be indicated as below.

Chloroform extract> Curcumin> Demethoxy curcumin> Bisdemethoxy curcumin

Summary

Curcuma longa L (fam.Zinggiberaceae), also known as turmeric, was highly esteemed by the ancient Indo-European people for its golden yellow dye resembling sunlight. This culture, known *as* Arya, worshipped the solar system and attributed special protective properties to those plants, which like turmeric contained sun coloured yellow dyes

(Wealth of India, 1950). It is having many medicinal properties and is used in folklore medicine for various ailments like asthma, rheumatism, swellings, antioxidants etc.

The whole plant of curcuma longa was used for the study. The chloroform extract of the plant rhizome was subjected to fractionation by column chromatography with solvents of graded polarity. A curcuminoid mixture fraction was obtained in the Chloroform: Methanol (95:5) fraction. This mixture was characterized by, thin layer chromatography and High performance Liquid chromatography. Again the mixture was purified with the 100% MDC (Methelene Dichloride) to isolate the main 3 curcuminoids i.e. Curcumin, Demethoxycurcumin, and Bisdemethoxycurumin. These compounds were characterized by, Thin Layer Chromatography and Spectral analysis (NMR, IR) (V.S. Govindarajan, 1988).

The in vitro pharmacological activates were performed for the chloroform extract and isolated compounds of the Curcuma Longa Rhizome

The chloroform extract and isolated compounds were evaluated for its anthelmintic activity. It showed dose dependant decreased in time taken for paralysis and death worms. The chloroform extract showed maximum activity compare to other extracts. The activity is comparable to that of standard. While comparing these isolated compounds with chloroform extract, the effect represented as below.

Chloroform extract> Curcumin> Demethoxy curcumin> Bisdemethoxy curcumin

Conclusion

From the above studies the chloroform extract and isolated compounds exhibited the anthelmitic activity the isolated molecules may be taken up for further studies to enable them to be explored as adjunts in anthelmintic therapy.

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Corresponding Author:

N.Sanjeevaiah*,

Email: *nagurla.sanjeev@gmail.com*